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Ecotoxicity evaluation of bromacil and tebuthiuron on native vegetation species in Alberta, Canada

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Soil sterilants such as bromacil and tebuthiuron were widely used across Alberta from the 1960s to the late 1990s. These non-selective, residual herbicides inhibit vegetation growth and pose risks for off-site migration. Current Alberta Tier one soil guidelines are primarily based on agronomic species, which may not reflect the sensitivity of native vegetation. This study evaluated the toxicity of bromacil and tebuthiuron to five native grass species, with four species tested for each sterilant, using a modified Environment Canada test protocol. Toxicity endpoints included emergence, root/shoot biomass, and root/shoot length, with inhibition concentration values derived for each species and endpoint. Species sensitivity distributions for the soil-applied herbicides bromacil and tebuthiuron were developed following the Canadian Council of Ministers of the Environment protocol to estimate effect concentrations for protection of the ecological direct contact pathway. Native species evaluated in the current studies exhibited greater sensitivity to bromacil and tebuthiuron than those assessed in earlier guideline development efforts. This study provides critical data to support the sustainable management of sterilant-impacted sites in Alberta, informing potential guideline development.

KEYWORDS

bromacil, herbicide, native species, soil sterilants, tebuthiuron, toxicity, vegetation

1 Introduction

Bromacil and tebuthiuron are persistent, non-selective herbicides that can prevent plant growth for extended periods (Dube et al., 2009; Troit and Sekwadi, 2012). While effective for weed control, there are concerns regarding their persistence in the environment (Dube et al., 2009). Bromacil and tebuthiuron were historically used on industrial sites and identified as two of the most used residual soil herbicides (commonly referred to as soil sterilants) in Alberta, Canada (Drozdowski et al., 2018b). In a 2018 Alberta stakeholder survey, bromacil was deemed the most problematic sterilant at 73%–91% of sites, with ~5% for tebuthiuron, compared to only <15% for others (e.g., atrazine) (Drozdowski et al., 2018a). Soil sterilants, such as tebuthiuron and bromacil, have historically been applied on rangelands, pipeline rights-of-way, oil and gas wells, railways, sawmills, pulp mills, and other industrial sites across Alberta (Powter and Drozdowski, 2024). Their extensive application is estimated to have affected over 60,000 sites across the province (Drozdowski et al., 2018a).

There is a need for cost-effective and sustainable management strategies to remediate sites contaminated with residual soil-applied herbicides, such as bromacil and tebuthiuron, particularly in non-agricultural and industrial settings. However, ecotoxicological data for

bromacil and tebuthiuron remain limited for plant species native to Alberta (Stantec Consulting Ltd, 2008). The current Alberta Tier 1 Soil and Groundwater Remediation Guidelines provide ecological direct contact benchmarks that are largely derived from toxicity data on agronomic species (Alberta Environment and Protected Areas, 2024; Alberta Environment and Protected Areas, 2024), such as durum wheat (*Triticum durum*) (Stantec Consulting Ltd, 2008) and alfalfa (Stantec Consulting Ltd, 2012). Existing literature presents mixed findings on whether native vegetation is more or less sensitive to herbicides compared to agronomic species. Some studies suggest that agronomic species may exhibit higher sensitivity than native vegetation adapted to local stressors (McKelvey et al., 2002). However, research on herbicide toxicity shows varied responses. For example, sulfonyleurea herbicide exhibited different phytotoxicity levels across plant species, with some native plants being as sensitive as crops (Olszyke et al., 2008), while some agronomic species showed tolerance to tebuthiuron (Pires et al., 2003). If native species are confirmed to be less sensitive, this differential tolerance could support the development of site-specific remedial endpoints for natural areas, where native vegetations are primary receptors assuming secondary exposure pathways are mitigated. Realizing this potential requires quantification of sterilant toxicity to ecologically relevant native Alberta species to recalibrate risk thresholds.

Therefore, this study evaluated the toxicity of soil sterilants bromacil and tebuthiuron on selected native plant species in Alberta, Canada. The findings are intended to support the development of science-based recommendations for direct soil eco-contact guidelines applicable to non-agricultural areas across the province. The research aligns with the broader objectives of Alberta's Soil Sterilants Program (Powter and Drozdowski, 2024), which aims to inform cost-effective and environmentally responsible practices for managing sites impacted by residual soil sterilants. By generating toxicity data specific to native species, this project contributes critical evidence for establishing soil concentration thresholds and guiding best management practices that minimize ecological risks. The study also generates species sensitivity distributions (SSDs) and derives effect concentrations.

2 Methods

2.1 Experimental species

Two separate species sets were used for toxicity testing of bromacil (*B. gracilis*, *Nassella viridula*, *Festuca hallii*, and *K. macrantha*) and tebuthiuron (*B. gracilis*, *N. viridula*, *Agropyron dasystachyum*, and *F. hallii*). The selected species are commonly used in reclamation efforts across Alberta, particularly in areas affected by soil sterilants (Powter et al., 2017). Locally adapted seeds were used whenever available. All seeds were wild collected within Alberta, except for *Bouteloua gracilis* and *Koeleria macrantha*, which were sourced commercially from Hannas Seeds (Lacombe, Alberta, Canada).

2.2 Test soil and chemicals

Loam soil was sourced from Brooks Asphalt and Aggregates (Brooks, Alberta), located within Alberta's Brown Soil Zone,

representative of soil type commonly impacted by soil sterilants. The physicochemical properties of the soil are listed in [Supplementary Table S1](#). The soil was homogenized using a large soil mixer (Bouldin & Lawson LLC, Tennessee, USA) and spiked with bromacil (Hyvar® X-L, liquid, 21.9% lithium salt) or tebuthiuron (Spike® 80DF, granular, 80% active ingredient) for the following toxicity tests. Bromacil was diluted in water to create stock solutions ranging from 0.01 to 100 g/L. The appropriate volume of solution was calculated based on the soil mass and poured into trenches formed in a subsample of soil. Tebuthiuron mass was weighed on a microbalance, dissolved in water, and similarly poured into soil trenches. Each subsample was mixed manually with a spatula, followed by mechanical mixing with an electric drill mixer until uniform. The spiked subsample was then combined with the remaining bulk soil and homogenized using the cone-and-quarter method (Schumacher et al., 1991), repeated a minimum of four times to ensure even distribution. Target concentrations (0.01–5 mg/kg for bromacil and 0.01–15 mg/kg for tebuthiuron) were informed by previous studies (Stantec Consulting Ltd, 2008; Stantec Consulting Ltd, 2012) and expert consultation (Personal Communication, G. Stephenson, 6 December 2019). Actual concentrations were verified via high-performance liquid chromatography/mass spectrometry (HPLC/MS) analysis (Element Materials Technology, Edmonton, Alberta), with detection limits of 0.0015 mg/kg for bromacil and 0.0012 mg/kg for tebuthiuron. Spiked soils were stored at 4 °C until toxicity testing. Where necessary, soils were re-spiked to meet target concentrations. Additional information on the spiking and homogenization process can be found in the previous report (Powter and Drozdowski, 2024).

2.3 Germination trial

Germination trials were conducted to assess seed viability and determine appropriate sowing methods. Seeds were planted in small pots and moistened to approximately 30% of the soil's water holding capacity, following Environment Canada (2007) guidelines (Environment Canada, 2007a). Pots were covered to maintain moisture, and germination was assessed after 14 days. Germination tests indicated that several species (e.g., *A. dasystachyum*, *P.smithii*, and *N. viridula*) required cold stratification. Despite stratification, germination rates for *B. gracilis* and *K. macrantha* remained low (<40%). Broadcast seeding was used for species with low germination rates—a method that inherently precludes measuring seedling emergence. Given that previous studies found emergence unaffected by these herbicides in some cases (Stantec Consulting Ltd, 2008; Stantec Consulting Ltd, 2012), emergence data were only collected for the range finding test (Section 2.4) and can be found elsewhere (Thacker, 2021); subsequent analyses focused exclusively on growth endpoints. For broadcast-seeded species, seedlings were thinned to 12 plants per pot after 2 weeks of growth.

2.4 Range finding test

Range-finding tests were conducted to identify appropriate concentrations of bromacil and tebuthiuron for subsequent definitive toxicity testing across selected native grass species.

These tests were used to define the concentration range of bromacil and tebuthiuron for the definitive toxicity test and detailed results are not included or discussed here (Environment Canada, 2007a).

2.5 Definitive toxicity test

An overview of the experimental design is provided in [Supplementary Table S2](#). The experiment was conducted in three trials, each involving two species. The experimental control was confirmed to be free of bromacil and tebuthiuron residues. Artificial soil was included for quality assurance purposes. Six replicates were conducted for the negative control and artificial soil, four replicates for the lowest six test concentrations, and three replicates for the highest five test concentrations.

Definitive toxicity testing was conducted following Environment Canada's (2007) protocol for terrestrial plant exposure to soil contaminants (Environment Canada, 2007a), with modifications to accommodate native species. Modifications included test duration, and no reference toxicity tests were conducted. Alberta native plant species tend to have slower growth rates than agronomic species; therefore, the test duration was extended from three to 6 weeks to accommodate their slower growth rates. It is acknowledged that not including a standard reference toxicant limits the ability to directly benchmark our test sensitivity against other labs or historic tests. Our priority was on testing numerous native species with limited seed availability and resources, focusing on guideline-relevant data.

One day before seeding, 1 L polypropylene pots were filled with approximately 0.5 L of soil. Water was added based on the moisture content of each sterilant-spiked soil to achieve a target gravimetric moisture of ~16%, equivalent to 30% of the soil's water holding capacity, consistent with the water holding capacity recommended by Environment Canada (Environment Canada, 2007a). Pots were randomly arranged in the greenhouse to minimize positional effects. Plants were grown in a controlled greenhouse environment. Conditions included a 16-h photoperiod and day/night temperatures of 24 °C/15 °C. Pots were covered for the first 7 days to maintain moisture and promote germination. After lid removal, plants were watered daily as needed using reverse osmosis water.

After 6 weeks of growth, plants were removed from the soil for root length, shoot length, and dry biomass measurement. The plants were removed by gently loosening and separating the roots from the soil by hand. The roots were then rinsed with water to remove any remaining soil. Root and shoot lengths were measured to the nearest millimetre using a ruler. Biomass samples were dried at 60 °C for 1 week and weighed using a precision balance (± 0.0001 g).

2.6 Data and statistical analysis

Data exploration, statistical analysis, and visualization were performed using the R programming language (The R Core Team, 2025) with the tidyverse package (Wickham et al., 2019). Pots with no germination were assigned NA values for root and shoot measurements, following Environment Canada guidance (Environment Canada, 2007b).

Effective concentration values for inhibition concentration (IC_{25}/IC_{50}) values for growth parameters were estimated using

the drc package (Ritz et al., 2015). IC_{25}/IC_{50} represent a 25%/50% reduction in growth from that of the control (Environment Canada, 2007a). Appropriate non-linear regression models were selected based on visual inspection of the data and statistical diagnostics, including the Shapiro-Wilk test for normality and Levene's test for homogeneity of variance. Where assumptions were violated, Box-Cox transformations were applied. In cases where transformations did not improve model fit, such as when replicate values were zero or limited, analysis proceeded with caution, and model robustness was evaluated based on fit diagnostics.

The species sensitivity distribution is generated by ranking the available IC_{25} (inhibition concentration) values (CCME, 2006). SSDs were generated for bromacil and tebuthiuron for three different datasets: i) all plant and soil invertebrate data, ii) native plants and soil invertebrate data, and iii) crop plants and soil invertebrate data. SSDs were constructed by plotting rank percentiles against measured soil concentrations of bromacil and tebuthiuron (rather than nominal values) using R (version 4.0.3) (Thorley and Schwarz, 2018). A logarithmic regression model was applied to the data to estimate the 25th and 50th percentile values of the distribution, referred to as the Estimated Species Sensitivity Distribution at the 25th percentile (ESSD₂₅) and 50th percentile (ESSD₅₀), respectively. This model was chosen for consistency with regulatory practice (Canadian Council of Ministers of the Environment, 2020) and because it provided a good fit to the historical dataset (Stantec Consulting Ltd, 2012). These ESSD values would be potential departure points for developing revised soil remediation guidelines. The uncertainty factor was set to 1, given the quality of the studies used. Full details are available in Litalien and Tindal (Litalien and Tindal, 2021).

3 Results

3.1 Bromacil and tebuthiuron concentrations in soil

Target and measured concentrations of bromacil and tebuthiuron in soil are summarized in [Supplementary Table S3](#). Most measured concentrations were similar or slightly lower than the target concentrations, with a few exceptions. All concentrations are reported on a dry weight basis. All details are provided in [Supplementary Material](#).

3.2 Ecotoxicity response and species sensitivity distributions for bromacil and tebuthiuron

Inhibition concentrations (IC_{50} and IC_{25}) calculated for each chemical, species, and endpoint are presented in [Table 1](#). The dose-response curve plots for each species and sterilant can be found from [Supplementary Figure S1-11](#). Generally, the IC_{25} and IC_{50} values for bromacil are lower than those for tebuthiuron ([Figure 1](#)).

SSDs for bromacil and tebuthiuron were constructed using the historical studies used to develop existing guidelines supplemented by ecotoxicological data from this study

TABLE 1 Estimated IC₅₀ and IC₂₅ values (mg/kg, length/dry weight basis) for shoot and root growth parameters across species exposed to bromacil and tebuthiuron. Standard errors (SE) are provided.

Species	Sterilant	Parameter	IC ₂₅	SE	IC ₅₀	SE
<i>Bouteloua gracilis</i>	Bromacil	Shoot length	0.1	0.01	0.18	0.01
		Root length	0.09	0.02	0.17	0.02
		Shoot weight	0.02	0.004	0.06	0.01
		Root weight	0.02	0.005	0.05	0.01
	Tebuthiuron	Shoot length	0.79	0.12	1.78	0.15
		Shoot weight	0.198	0.037	0.406	0.092
Root weight		0.275	0.057	0.476	0.079	
<i>Koeleria macrantha</i>	Bromacil	Shoot length	0.013	0.004	0.038	0.008
		Root length	0.031	0.002	0.033	0.001
		Shoot weight	0.006	0.003	0.013	0.004
		Root weight	0.004	0.001	0.011	0.002
<i>Nassella viridula</i>	Bromacil	Shoot length	0.023	0.003	0.033	0.003
		Shoot weight	0.01	0.002	0.015	0.002
		Root weight	0.01	0.001	0.014	0.001
	Tebuthiuron	Shoot length	0.089	0.014	0.142	0.015
		Root length	0.12	0.011	0.151	0.015
		Shoot weight	0.033	0.007	0.053	0.007
Root weight		0.03	0.006	0.039	0.006	
<i>Agropyron dasystachyum</i>	Tebuthiuron	Root length	0.108	0.015	0.128	0.01
		Shoot weight	0.012	0.003	0.03	0.006
		Root weight	0.026	0.017	0.04	0.011
<i>Festuca hallii</i>	Bromacil	Shoot length	0.023	0.005	0.045	0.009
		Root length	0.01	0.002	0.017	0.002
		Shoot weight	0.017	0.005	0.025	0.006
		Root weight	0.015	0.005	0.021	0.004
	Tebuthiuron	Shoot length	0.116	0.029	0.208	0.06
		Shoot weight	0.086	0.026	0.104	0.018
Root weight		0.017	0.006	0.041	0.015	

(Figure 2). The fitted regression models demonstrated generally good statistical fit across species. Among the tested organisms, native plant species, including *K. macrantha*, *Festuca hallii*, and *N. viridula*, exhibited the highest sensitivity to bromacil. *F. hallii*, *A. dasystachyum*, and *N. viridula* were among the most sensitive to tebuthiuron. SSDs for bromacil and tebuthiuron were also constructed for two subsets of the full dataset: native plants plus all invertebrates, and crop plants plus all invertebrates. These distributions are provided in Supplementary Figures S12, S13, respectively. The Estimated Species Sensitivity Distribution at the 25th percentile (ESSD₂₅) and 50th percentile (ESSD₅₀) for each of these six SSDs are summarized in Table 2.

4 Discussion

The primary objective of this study was to expand the toxicological dataset used to inform potential soil remediation guidelines for bromacil and tebuthiuron, specifically for the ecological direct contact exposure pathway in fine-grained soil. The work reported herein has added data for four native grass species for each of these chemicals. Our findings demonstrate that several Alberta native plant species are sensitive to the soil-applied herbicides bromacil and tebuthiuron. These findings align with the broader context of herbicide ecotoxicology, which suggests that non-target, native plants can be affected by herbicide residues (Woody-Pumford et al., 2025).

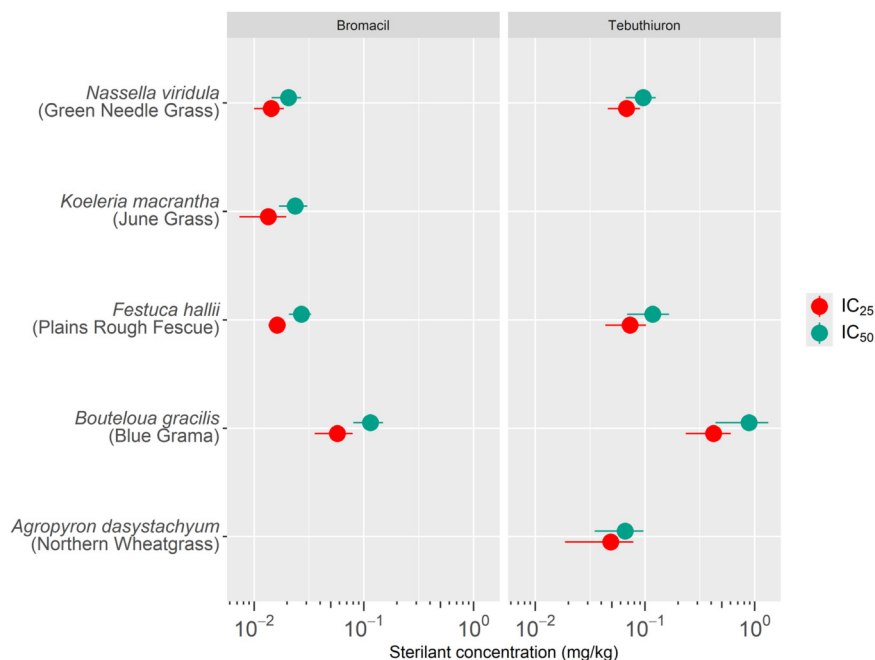


FIGURE 1
IC₂₅ and IC₅₀ values using measured concentrations, averaged across shoot length, root length, shoot weight and root weight for each species, sterilant and sterilant concentration (mg/kg). Error bars represent the standard error of the mean.

The newly collected ecological data were combined with the existing data to generate SSDs for bromacil and tebuthiuron, including datasets for all species, native plants only plus invertebrates, and crop plants only plus invertebrates. These six SSDs are presented in [Figure 1](#) and [Supplementary Figures S12, S13](#). The 25th and 50th percentiles of each distribution are summarized in [Table 2](#) and would be a point of departure for potential future guideline development.

Clark et al. emphasized that no single species or endpoint consistently represents maximum sensitivity across all chemicals ([Clark et al., 2004](#)), indicating that the relative sensitivity of native and agronomic plants is chemical- and species-specific. Comparing the ESSD₂₅ and ESSD₅₀ values for native plants and invertebrates in [Table 2](#) with those for crop plants and invertebrates makes it clear that the native plants included in the current study are an order of magnitude more sensitive to both bromacil and tebuthiuron than are the crop plants used to develop the existing Alberta Tier guidelines for fine-grained soils.

Several factors could explain why native species showed greater sensitivity in our tests. The extended 6-week growth period used in this study (vs. 21 days in previous studies) allowed for a more comprehensive assessment of toxicity, particularly for slow-growing native species. It is not unexpected that toxicity increases with exposure duration. Studies on triazine herbicides showed higher long-term toxicity compared to short-term effects for some compounds ([Zhu et al., 2009](#)). A study on periphyton communities showed that herbicide effects intensified with increased exposure time, with IC₅₀ values decreasing by one to two orders of magnitude as exposure increased from 1 to 24 h ([Gustavson et al., 2003](#)). The current study used Hyvar[®] X-L, a liquid formulation containing 78.6% inert solvents (ethanediol, ethanol, methanol, lithium hydroxide) ([Bayer Environmental](#)

[Science, 2016](#)). In contrast, Stantec used DuPont[™] Hyvar[®] X, composed of only 20% other ingredients (e.g., <1% quartz) without these additives (80% bromacil) ([Stantec Consulting Ltd, 2012](#)). In addition, differences in soil properties may also have influenced toxicity results. Literature indicates that bromacil's phytotoxicity varies among plant species and can be influenced by soil amendments ([El-Nahhal and Hamdona, 2017](#)). Although precise soil classification was not possible in the current study due to the removal of topsoil, two soil pits were excavated in adjacent undisturbed areas to support classification efforts. Based on regional soil mapping data ([Government of Alberta, 2026](#)), field observations from the soil pits ([Supplementary Figure S14](#)), and laboratory analyses ([Supplementary Table S1](#)), the soil used was most likely classified as either an Orthic Brown Chernozem or a Solonetzic Brown Chernozem. Thus, the sensitivity we observed in native species may be partly a function of both chemical/biological traits and the specific soil conditions.

This difference in sensitivity could potentially be utilized in risk management efforts at sites with residual bromacil and tebuthiuron concentrations to better understand how crop species and native grasses may differ in their responses to residual levels of these herbicides. Our results align with broader ecotoxicology principles, which suggest that different plant species (even within the same functional group) can vary by an order of magnitude or more in their response to a given toxicant ([Spurgeon et al., 2020](#)). In addition, our findings provide evidence that using only crop surrogates in phytotoxicity tests may under-predict risks to wild plant communities. For instance, in a 15-species greenhouse trial, it was found that wild non-crop plants were "overall more sensitive" to herbicides than the species in the US EPA's crop-based dataset ([Boutin et al., 2004](#)). Our results highlight that standard test species

TABLE 2 Derived effects concentrations (ESSD₂₅ and ESSD₅₀ Values).

Sterilant	Dataset	ESSD ₂₅ (mg/kg)	ESSD ₅₀ (mg/kg)	n studies	n independent data points
Bromacil	All data	0.028	0.21	2	29
	Native plants and invertebrates	0.014	0.14	2	20
	Crop plants and invertebrates	0.37	2.2	1	13
Tebuthiuron	All data	0.018	0.15	2	28
	Native plants and invertebrates	0.023	0.12	2	24
	Crop plants and invertebrates	0.56	4.6	1	7

and recommends further research on soil type variability, particularly for coarse-textured soils, to refine management strategies for sterilant-impacted sites (Parven et al., 2025).

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Author contributions

ST: Data curation, Investigation, Methodology, Project administration, Resources, Writing – original draft. MT: Investigation, Methodology, Validation, Writing – review and editing. BD: Conceptualization, Funding acquisition, Resources, Supervision, Writing – review and editing. VB: Resources, Writing – review and editing. SS: Formal Analysis, Software, Visualization, Writing – review and editing. ZC: Funding acquisition, Validation, Writing – original draft.

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Conflict of interest

Author MT was employed by Millennium EMS Solutions Ltd.. Authors ST, BD, VB, SS, and ZC were employees of InnoTech Alberta at the time of the study.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fenvs.2026.1745999/full#supplementary-material>

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